# PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

## PCT

### NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE
in its capacity as elected Office

| | |
|---|---|
| **Date of mailing** (day/month/year)<br>28 May 2001 (28.05.01) | |
| **International application No.**<br>PCT/SE00/01767 | **Applicant's or agent's file reference**<br>192929501/BN |
| **International filing date** (day/month/year)<br>13 September 2000 (13.09.00) | **Priority date** (day/month/year)<br>17 September 1999 (17.09.99) |

**Applicant**

HÖGLUND, Anna-Stina et al

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

04 April 2001 (04.04.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

| | |
|---|---|
| The International Bureau of WIPO<br>34, chemin des Colombettes<br>1211 Geneva 20, Switzerland | Authorized officer<br>Nestor Santesso |
| Facsimile No.: (41-22) 740.14.35 | Telephone No.: (41-22) 338.83.38 |

PATENT COOPERATION TREATY

# PCT



## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

12

| Applicant's or agent's file reference | FOR FURTHER ACTION | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| 192929501/BLN | | |
| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
| PCT/SE00/01767 | 13.09.2000 | 17.09.1999 |

International Patent Classification (IPC) or national classification and IPC7

C12N 15/82, C12N 9/02, C12N 9/10, A01H 5/00, C12P 23/00 // (C12N 9/02, C12R 1:89

Applicant

AstaCarotene AB et al

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 1 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 04.04.2001 | 14.12.2001 |
| Name and mailing address of the IPEA/SE | Authorized officer |
| Patent- och registreringsverket<br>Box 5055<br>S-102 42 STOCKHOLM<br>Facsimile No. 08-667 72 88 | Telex<br>17978<br>PATOREG-S |
| | Fernando Farieta/EÖ<br>Telephone No. 08-782 25 00 |

Form PCT/IPEA/409 (cover sheet) (January 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/SE00/01767

## I. Basis of the report

1. With regard to the **elements** of the international application:*

- ☐ the international application as originally filed
- ☒ the description:
  - pages 1-7, as originally filed
  - pages _____, filed with the demand
  - pages _____, filed with the letter of _____
- ☒ the claims:
  - pages _____, as originally filed
  - pages _____, as amended (together with any statement) under article 19
  - pages _____, filed with the demand
  - pages 1, filed with the letter of 12.11.2001
- ☒ the drawings:
  - pages --, as originally filed
  - pages _____, filed with the demand
  - pages _____, filed with the letter of _____
- ☒ the sequence listing part of the description:
  - pages 1-5, as originally filed
  - pages _____, filed with the demand
  - pages _____, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages _____
   - ☐ the claims, Nos. _____
   - ☐ the drawings, sheet/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2 (c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item I and annexed to this report.*

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Claims | 1-9 | YES |
| | Claims | | NO |
| Inventive step (IS) | Claims | 1-9 | YES |
| | Claims | | NO |
| Industrial applicability (IA) | Claims | 1-9 | YES |
| | Claims | | NO |

2. Citations and explanations (Rule 70.7)

The claimed invention relates to transgenic oilseed plant cells containing a DNA construct, wherein the cell expresses xanthophylls.

The amended claims filed with the letter of 2001-11-12 differ from the claims as originally filed. Claim 1 is based on the original claims 1, 2, 4 and 6, and the sub-claims have all characteristics appearing in the original set of claims and page 2, lines 5-9 of the description. Thus, the invention fulfils the requirement of unity.

A description of experiments with the heading "Keto-carotenoids in transgenic plants" and containing two figures, is also enclosed. In this opinion the following documents are cited:

D1 : WO9907867 A1
D2 : WO9806862 A1
D3 : WO9818910 A1

**Document D1** discloses methods for producing plants and seeds having altered carotenoid compositions by transforming host plants with constructs, genes and coding regions. (page 13 line 15-23). The methods find particular use in increasing the carotenoid content in oilseed plants. The method comprises the steps of: transforming the cells, producing a transformed host plant and growing said transformed host plant under conditions whereby seed is produced having an altered xanthophyll content (claims 1-4). Other components in the method are: a transcriptional initiation region from a gene, a plastid transit peptide, a DNA sequence derived from a carotenoid biosynthesis gene coding region, and a transcriptional termination region, producing tranformed cells, growing progeny thereof containing said construct for producing xanthophylls. No production of astaxanthin is shown in the examples.

.../...

**Supplemental Box**
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: V

**Document D2** concerns the production of a seed having an increase in carotenoid biosynthesis, transformation of the plant (page 8 lines 9-12). Several genes encode for the production of canthaxantin according to documents named as the prior art (WO95/18220 or WO96/06172); and genes as crtZ, crtW and crtO for the production of astaxanthin (page 12 lines 12-15). Any means for producing a plant comprising the primary gene of both the primary and secondary genes are encompassed by document D2. Napin (page 14 line 22-25) is suggested as a promotor in nopaline synthase termination regions. In the experimental part of D2 there are no analysed xanthophylls except for lutein and cryptoxanthin in the tables.
Document D2 comprises: " a method for altering carotenoid levels in a seed from a host plant, said method comprising transforming, said host plant with a construct comprising as *operably linked components*, a transcriptional initiation region from a gene, a *plastid transit* peptide, a *DNA coding sequence* and a *transcriptional termination region* ". Claim 27 refers to the use of napin gene and claims 61 and 66 concern canthaxanthin and astaxanthin.

**Document D3** discloses a nucleic acid sequence encoding beta-C-4-oxygenase from *Haematococcus Pluvialis* for the biosynthesis of astaxanthin, including the crtO gene nucleic and corresponding polypeptide sequences, allelic and species variants or functional naturally occurring or man-induced variants thereof (page 25 lines 19-32). Thus, the only construct comprising a nucleic acid sequence encoding a beta-C4-oxygenase is the disclosed *Haematococcus pluvalis crtO* gene.

In document D3, the DNA segment is present in the vector operably linked to a promoter (page 27 line 21). The carotenoid content is analysed from leaves and nectary tissue of flowers. A transit peptide is linked to the coding sequence of crtO from *H. Pluvialis* (page 39 line 18). Claims 28-33 concerns transgenic plant chloroplasts or chromoplasts. Various glycosilated carotenoids and carotenoids esters have been identified (page 6 lines 18-23).

.../...

**Supplemental Box**
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: V

Conclusion

Claims 1-9

In the claimed invention there is not needed any gene in the expression cassette coding for beta-carotene hydroxylase to produce astaxanthin and cantaxanthin. The claimed invention uses a construct with a 5'-truncated variant of beta-C4-oxygenase, the truncation is not defined in the description. The DNA construct is defined as SEQ ID No1.(Claim 4).

The nucleotide sequence coding for "a transit peptide directing the translated fusion polypeptide to the chloroplast of the oilseed plant" is considered to be a new feature in the art.
It is considered that a person skilled in the art would not try to produce a transgenic oilseed plant cell containing the following technical features:

- A DNA construct comprising in the 5' to 3' direction of
- A transcription operably linked promoter region,
- A nucleotide sequence coding for a transit peptide directing the translated fusion polypeptide to the chloroplast.
- A 5'-truncated variant of beta-C4-oxygenase,
- A transcriptional termination region.

For a man skilled in the art is not obvious that such combination will have a reasonable expectation of success. Documents D1-D3 are considered to represent the prior art. Therefore, claims 1-9 are considered to fulfil the requirements of industrial applicability (IA), novelty (N) and inventive step (IS).

## Claims

1. Transgenic oilseed plant cell containing a DNA construct comprising in the 5′ to 3′ direction of transcription operably linked a promoter region directing transcription to the seed of the oilseed plant, a nucleotide sequence coding for a transit peptide directing the translated fusion polypeptide to the chloroplast of the oilseed plant, a 5'-truncated beta-carotene C-4-oxygenase gene from the alga *Haematococcus pluvialis* and a transcriptional termination region.

2. Transgenic oilseed plant cell according to claim 1, wherein the cell additionally contains at least one DNA construct selected from DNA constructs comprising in the 5′ to 3′ direction of transcription operably linked a promoter region directing transcription to the seed of the oilseed plant, a nucleotide sequence coding for a transit peptide directing the translated fusion polypeptide to the chloroplast of the oilseed plant, a nucleotide sequence coding for at least one peptide with enzyme activity necessary for keto group containing xanthophyll production and esterification in the oilseed plant and a transcriptional termination region.

3. Transgenic oilseed plant cell according to claim 1 or 2, wherein the promoter is a napin promoter, the peptide with enzyme activity necessary for keto group containing xanthophyll production and esterification is selected from the group consisting of peptides with, 1-D-deoxyxylulose 5-phosphate synthase, isopentenyl pyrophosphate:dimethylallyl pyrophosphate isomerase, geranylgeranyl pyrophosphate synthase, phytoene synthase, phytoene desaturase, zeta-carotene desaturase, lycopene beta-cyclase, β-carotene hydroxylase, and acyl transferase activity.

4. Transgenic oilseed plant cell according to claim 1, wherein the nucleotide sequence of the DNA construct is SEQ ID NO:1.

5. Transgenic oilseed plant cell according to any one of claims 1 - 5, wherein the oilseed plant is selected from the group consisting of rape, sunflower, soybean and mustard.

6. Transgenic oilseed plant cell according to any one of claims 1 – 5, wherein the cell expresses xanthophylls.

7. Transgenic oilseed plant cell according to claim 6, wherein a xanthophyll is canthaxanthin.

8. Transgenic oilseed plant cell according to claim 6, wherein a xanthophyll is astaxanthin.

9. Transgenic oilseed plant cell according to claim 8, wherein the astaxanthin comprises astaxanthin esters.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 00/01767

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C12N 15/82, C12N 9/02, C12N 9/10, A01H 5/00, C12P 23/00 // (C12N 9/02, C12R 1:89)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C12N, C12P, A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 9907867 A1 (CALGENE LLC), 18 February 1999 (18.02.99), see abstract, page 13, lines 15-23, claims<br>-- | 1-11 |
| X | WO 9806862 A1 (CALGENE, INC.), 19 February 1998 (19.02.98), see page 8. line 9 - page 12, line 15; page 13, line 22 - page 15, line 5<br>-- | 1-11 |
| X | Susan Budavari et al "THE MERCK INDEX", twelfth edition", 1996, MERCK & CO., INC. NJ, see entries 890, "Astaxanthin"; 1798, "Canthaxanthin"; 10197, "Xanthophyll".<br>-- | 8-10 |

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 12 December 2000 | - 20 -12- 2000 |
| Name and mailing address of the ISA/<br>Swedish Patent Office<br>Box 5055, S-102 42 STOCKHOLM<br>Facsimile No. +46 8 666 02 86 | Authorized officer<br>Hampus Rystedt/GH<br>Telephone No. +46 8 782 25 00 |

Form PCT/ISA/210 (second sheet) (July 1998)

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 9818910 A1 (YISSUM RESEARCH AND DEVELOPMENT COMPANY OF THE HEBREW UNIVERSITY OF JERUSALEM), 7 May 1998 (07.05.98), see abstract, page 28, line 24 - page 29, line 4 | 1-4 |
| A | -- | 5-11 |
| A | WO 9613149 A1 (AMOCO CORPORATION), 9 May 1996 (09.05.96) | 1-11 |
| | -- | |
| A | EMBL/GenBank/DDBJ databases, accession no. X86782, 1997-11-30, Harker M. et al: "H.pluvialis mRNA for beta-carotene C-4 oxygenase" | 4,5 |
| | -- | |
| A | EMBL/GenBank/DDBJ databases, accession no. D45881, 1995-12-29, Kajiwarea S.: "Haematococcus pluvialis mRNA for bet-carotene ketolase, complete cds" | 3 |
| | -- | |
| A | EMBL/GenBank/DDBJ databases, accession no. X86783, 1998-06-02, Harker M. et al: "H.pluvialis mRNA for phyteone desaturase" | 3 |
| | -- | |
| A | EMBL/GenBank/DDBJ databases, accession no. AF082325, Sun Z. et al: "Haematococcus pluvialis isopentenyl pyrophosphate:dimethylallyl pyrophosphate isomerase (ipiHp1) mRNA, complete cd, 1998-08-18 | 3 |
| | -- | |
| X | EMBL/GenBank/DDBJ databases, accession no. AF082326, 1998-08-18, Sun Z. et al: "Haematococcus pluvialis isopenetyl pyrophosphate:dimethylallyl pyrophosphate isomerase (ipiHp2) mRNA, complete cds" | 3 |
| | -- | |

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | EMBL/GenBank/DDBJ databases, accession no. AF162276, 1999-09-10, Linden H.: "Haematococcus pluvialis carotenoid hydroxylase mRNA, partial cds" -- | 3 |
| A | WO 9930701 A1 (ASTACAROTENE), 24 June 1999 (24.06.99), see abstract and claims -- | 11 |
| A | WO 9837874 A1 (ASTACAROTENE AB), 3 Sept 1998 (03.09.98), see abstract and claims -- | 11 |
| A | JOURNAL OF PHOTOCHEMISTRY AND PHOTOBIOLOGY B, Volume 30, 1995, BISWAL, B et al, "Carotenoid catabolism durint leaf senescence and its control by light" page 3 - page 13 -- -------- | 11 |

INTERNATIONAL SEARCH REPORT

International application No.
SE00/01767

**Box I** **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II** **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

see extra sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

According to Article 34 (3) (a-c) and Rule 13.2, an international application shall relate to one invention only or to a group of inventions linked by one or more of the same or corresponding "special technical features", i.e. features that define a contribution which each of the inventions makes over the prior art. The present application relates to five such groups of inventions, namely:

1. A DNA construct encoding an enzyme in the carotenoid biosynthetic pathway and cells expressing the enzyme, according to claims 1-7.
2. Transgenic oilseed plant-produced xanthophyll, according to claim 8.
3. Transgenic oilseed plant-produced canthaxanthin, according to claim 9.
4. Transgenic oilseed plant-produced astaxanthin, according to claim 10.
5. Transgenic oilseed plant-produced astaxanthin esters, according to claim 11.

The feature common to all inventions is the transgenic production of carotenoids in oilseed plants. However, this feature is already known through WO-A1-9806862. The production of different carotenoids, and DNA constructs facilitating the production, is thus not linked by a special technical feature as required by Rule 13.2. As the additional effort of searching inventions 2-5 does not justify an additional search fee, all inventions have been searched.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/SE 00/01767

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 9907867 A1 | 18/02/99 | AU 8900298 A | 01/03/99 |
| | | EP 1002117 A | 24/05/00 |
| WO 9806862 A1 | 19/02/98 | AU 4058497 A | 06/03/98 |
| | | BR 9713462 A | 28/03/00 |
| | | CN 1227609 A | 01/09/99 |
| | | EP 0925366 A | 30/06/99 |
| WO 9818910 A1 | 07/05/98 | AU 4743697 A | 22/05/98 |
| | | NO 991996 A | 22/06/99 |
| | | US 5916791 A | 29/06/99 |
| | | US 5965795 A | 12/10/99 |
| | | CN 1247565 A | 15/03/00 |
| | | EP 0951534 A | 27/10/99 |
| | | PL 332965 A | 25/10/99 |
| WO 9613149 A1 | 09/05/96 | AU 697358 B | 01/10/98 |
| | | AU 3970195 A | 23/05/96 |
| | | CA 2203815 A | 09/05/96 |
| | | CN 1172416 A | 04/02/98 |
| | | EP 0792352 A | 03/09/97 |
| | | JP 10509309 T | 14/09/98 |
| | | NO 971945 A | 27/06/97 |
| | | NZ 296012 A | 28/05/99 |
| | | PL 319788 A | 01/09/97 |
| | | US 5618988 A | 08/04/97 |
| WO 9930701 A1 | 24/06/99 | AU 1897299 A | 05/07/99 |
| | | EP 1049460 A | 08/11/00 |
| | | NO 20003042 A | 14/06/00 |
| | | SE 511237 C | 30/08/99 |
| | | SE 9704693 A | 17/06/99 |
| WO 9837874 A1 | 03/09/98 | AU 719090 B | 04/05/00 |
| | | AU 2796797 A | 19/11/97 |
| | | AU 6295198 A | 18/09/98 |
| | | CN 1248912 T | 29/03/00 |
| | | EP 0898823 A | 03/03/99 |
| | | EP 0981338 A | 01/03/00 |
| | | NO 994109 A | 27/10/99 |
| | | PL 335370 A | 25/04/00 |
| | | SE 9700708 A | 28/08/98 |

# INTERNATIONAL-TYPE SEARCH REPORT

Search request No.
[redacted]99/01243

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C12N 15/82, C12N 9/02, C12N 9/10, A01H 5/00, C12P 23/00 // (C12N 9/02, C12R 1:89)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C12N, C12P, A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 9907867 A1 (CALGENE LLC), 18 February 1999 (18.02.99), see abstract, page 13, lines 15-23, claims<br>-- | 1-11 |
| X | WO 9806862 A1 (CALGENE, INC.), 19 February 1998 (19.02.98), see page 8, line 9 - page 12, line 15, page 13, line 22 - page 15, line 5<br>-- | 1-11 |
| A | WO 9818910 A1 (YISSUM RESEARCH AND DEVELOPMENT COMPANY OF THE HEBREW UNIVERSITY OF JERUSALEM), 7 May 1998 (07.05.98), see abstract, page 28, line 24 - page 29, line 4 | 1-4 |
| A | -- | 5-11 |

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international-type search | Date of mailing of the international-tupe search report |
|---|---|
| 13 April 2000 | 2000 -06- 07 |
| Name and mailing address of the ISA/<br>Swedish Patent Office<br>Box 5055, S-102 42 STOCKHOLM<br>Facsimile No. +46 8 666 02 86 | Authorized officer<br>Hampus Rystedt/EÖ<br>Telephone No. +46 8 782 25 00 |

INTERNATIONAL-TYPE SEARCH REPORT

Search request No. 99/01243

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | WO 9613149 A1 (AMOCO CORPORATION), 9 May 1996 (09.05.96) | 1-11 |
| | -- | |
| A | EMBL/GenBank/DDBJ databases, accession no. X86782, 1997-11-30, Harker M. et al: ""H.pluvialis mRNA for beta-carotene C-4 oxygenase" | 4,5 |
| | -- | |
| A | EMBL/GenBank/DDBJ databases, accession no. D45881, 1995-12-29, Kajiwarea S.: "Haematococcus pluvialis mRNA for beta-carotene ketolase, complete cds" | 3 |
| | -- | |
| A | EMBL/GenBank/DDBJ databases, accession no. X86783, 1998-06-02, Harker M. et al: "H.pluvialis mRNA for phytoene desaturase" | 3 |
| | -- | |
| A | EMBL/GenBank/DDBJ databases, accession no. AF082325, Sun Z. et al: "Haematococcus pluvialis isopentenyl pyrophosphate:dimethylallyl pyrophosphate isomerase (ipiHp1) mRNA, complete cd, 1998-08-18 | 3 |
| | -- | |
| A | EMBL/GenBank/DDBJ databases, accession no. AF082326, 1998-08-18, Sun Z. et al: "Haematococcus pluvialis isopentenyl pyrophosphate:dimethylallyl pyrophosphate isomerase (ipiHp2) mRNA, complete cds" | 3 |
| | -- | |
| A | EMBL/GenBank/DDBJ databases, accession no. AF162276, 1999-09-10, Linden H.: "Haematococcus pluvialis carotenoid hydroxylase mRNA, partial cds" | 3 |
| | -- | |
| A | WO 9930701 A1 (ASTACAROTENE AB), 24 June 1999 (24.06.99), see abstract and claims | 11 |
| | -- | |

INTERNATIONAL-TYPE SEARCH REPORT

Search request No.
99/01243

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | WO 9837874 A1 (ASTACAROTENE AB), 3 September 1998 (03.09.98), see abstract and claims<br><br>-- | 11 |
| A | Dialog Information Services, File 34, SciSearch, Dialog accession no. 04511134, BISWAL B: "Carotenoid catabolism during leaf senescence and its control by light"; & Journal of photochemistry and photobiology B-biology, 1995, v30 N1 (SEP), P3-13<br><br>--<br><br>-------- | 11 |

| | | | | | | |
|---|---|---|---|---|---|---|
| WO | 9907867 | A1 | 18/02/99 | AU | 8900298 | A 01/03/99 |
| WO | 9806862 | A1 | 19/02/98 | AU | 4058497 | A 06/03/98 |
| | | | | CN | 1227609 | A 01/09/99 |
| | | | | EP | 0925366 | A 30/06/99 |
| WO | 9818910 | A1 | 07/05/98 | AU | 4743697 | A 22/05/98 |
| | | | | EP | 0951534 | A 27/10/99 |
| | | | | NO | 991996 | A 22/06/99 |
| | | | | US | 5916791 | A 29/06/99 |
| | | | | US | 5965795 | A 12/10/99 |
| WO | 9613149 | A1 | 09/05/96 | AU | 697358 | B 01/10/98 |
| | | | | AU | 3970195 | A 23/05/96 |
| | | | | CA | 2203815 | A 09/05/96 |
| | | | | CN | 1172416 | A 04/02/98 |
| | | | | EP | 0792352 | A 03/09/97 |
| | | | | JP | 10509309 | T 14/09/98 |
| | | | | NO | 971945 | A 27/06/97 |
| | | | | NZ | 296012 | A 28/05/99 |
| | | | | PL | 319788 | A 01/09/97 |
| | | | | US | 5618988 | A 08/04/97 |
| WO | 9930701 | A1 | 24/06/99 | AU | 1897299 | A 05/07/99 |
| | | | | SE | 511237 | C 30/08/99 |
| | | | | SE | 9704693 | A 17/06/99 |
| WO | 9837874 | A1 | 03/09/98 | AU | 2796797 | A 19/11/97 |
| | | | | AU | 6295198 | A 18/09/98 |
| | | | | EP | 0898823 | A 03/03/99 |
| | | | | NO | 994109 | D 00/00/00 |
| | | | | SE | 9700708 | A 28/08/98 |

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 00/01767

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C12N 15/82, C12N 9/02, C12N 9/10, A01H 5/00, C12P 23/00 // (C12N 9/02, C12R 1:89)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C12N, C12P, A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 9907867 A1 (CALGENE LLC), 18 February 1999 (18.02.99), see abstract, page 13, lines 15-23, claims<br>-- | 1-11 |
| X | WO 9806862 A1 (CALGENE, INC.), 19 February 1998 (19.02.98), see page 8, line 9 - page 12, line 15; page 13, line 22 - page 15, line 5<br>-- | 1-11 |
| X | Susan Budavari et al "THE MERCK INDEX", twelfth edition", 1996, MERCK & CO., INC. NJ, see entries 890, "Astaxanthin"; 1798, "Canthaxanthin"; 10197, "Xanthophyll".<br>-- | 8-10 |

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 12 December 2000 | - 20 -12- 2000 |
| Name and mailing address of the ISA/<br>Swedish Patent Office<br>Box 5055, S-102 42 STOCKHOLM<br>Facsimile No. +46 8 666 02 86 | Authorized officer<br>Hampus Rystedt/GH<br>Telephone No. +46 8 782 25 00 |

Form PCT/ISA/210 (second sheet) (July 1998)

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 9818910 A1 (YISSUM RESEARCH AND DEVELOPMENT COMPANY OF THE HEBREW UNIVERSITY OF JERUSALEM), 7 May 1998 (07.05.98), see abstract, page 28, line 24 - page 29, line 4 | 1-4 |
| A | -- | 5-11 |
| A | WO 9613149 A1 (AMOCO CORPORATION), 9 May 1996 (09.05.96) | 1-11 |
| | -- | |
| A | EMBL/GenBank/DDBJ databases, accession no. X86782, 1997-11-30, Harker M. et al: "H.pluvialis mRNA for beta-carotene C-4 oxygenase" | 4,5 |
| | -- | |
| A | EMBL/GenBank/DDBJ databases, accession no. D45881, 1995-12-29, Kajiwarea S.: "Haematococcus pluvialis mRNA for bet-carotene ketolase, complete cds" | 3 |
| | -- | |
| A | EMBL/GenBank/DDBJ databases, accession no. X86783, 1998-06-02, Harker M. et al: "H.pluvialis mRNA for phyteone desaturase" | 3 |
| | -- | |
| A | EMBL/GenBank/DDBJ databases, accession no. AF082325, Sun Z. et al: "Haematococcus pluvialis isopentenyl pyrophosphate:dimethylallyl pyrophosphate isomerase (ipiHp1) mRNA, complete cd, 1998-08-18 | 3 |
| | -- | |
| X | EMBL/GenBank/DDBJ databases, accession no. AF082326, 1998-08-18, Sun Z. et al: "Haematococcus pluvialis isopenetyl pyrophosphate:dimethylallyl pyrophosphate isomerase (ipiHp2) mRNA, complete cds" | 3 |
| | -- | |

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | EMBL/GenBank/DDBJ databases, accession no. AF162276, 1999-09-10, Linden H.: "Haematococcus pluvialis carotenoid hydroxylase mRNA, partial cds" -- | 3 |
| A | WO 9930701 A1 (ASTACAROTENE), 24 June 1999 (24.06.99), see abstract and claims -- | 11 |
| A | WO 9837874 A1 (ASTACAROTENE AB), 3 Sept 1998 (03.09.98), see abstract and claims -- | 11 |
| A | JOURNAL OF PHOTOCHEMISTRY AND PHOTOBIOLOGY B, Volume 30, 1995, BISWAL, B et al, "Carotenoid catabolism durint leaf senescence and its control by light" page 3 - page 13 -- | 11 |

---

INTERNATIONAL SEARCH REPORT

International application No.
S[redacted]/01767

**Box I** **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II** **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

see extra sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

According to Article 34 (3) (a-c) and Rule 13.2, an international application shall relate to one invention only or to a group of inventions linked by one or more of the same or corresponding "special technical features", i.e. features that define a contribution which each of the inventions makes over the prior art. The present application relates to five such groups of inventions, namely:

1. A DNA construct encoding an enzyme in the carotenoid biosynthetic pathway and cells expressing the enzyme, according to claims 1-7.
2. Transgenic oilseed plant-produced xanthophyll, according to claim 8.
3. Transgenic oilseed plant-produced canthaxanthin, according to claim 9.
4. Transgenic oilseed plant-produced astaxanthin, according to claim 10.
5. Transgenic oilseed plant-produced astaxanthin esters, according to claim 11.

The feature common to all inventions is the transgenic production of carotenoids in oilseed plants. However, this feature is already known through WO-A1-9806862. The production of different carotenoids, and DNA constructs facilitating the production, is thus not linked by a special technical feature as required by Rule 13.2. As the additional effort of searching inventions 2-5 does not justify an additional search fee, all inventions have been searched.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/SE 00/01767

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 9907867 A1 | 18/02/99 | AU 8900298 A | 01/03/99 |
| | | EP 1002117 A | 24/05/00 |
| WO 9806862 A1 | 19/02/98 | AU 4058497 A | 06/03/98 |
| | | BR 9713462 A | 28/03/00 |
| | | CN 1227609 A | 01/09/99 |
| | | EP 0925366 A | 30/06/99 |
| WO 9818910 A1 | 07/05/98 | AU 4743697 A | 22/05/98 |
| | | NO 991996 A | 22/06/99 |
| | | US 5916791 A | 29/06/99 |
| | | US 5965795 A | 12/10/99 |
| | | CN 1247565 A | 15/03/00 |
| | | EP 0951534 A | 27/10/99 |
| | | PL 332965 A | 25/10/99 |
| WO 9613149 A1 | 09/05/96 | AU 697358 B | 01/10/98 |
| | | AU 3970195 A | 23/05/96 |
| | | CA 2203815 A | 09/05/96 |
| | | CN 1172416 A | 04/02/98 |
| | | EP 0792352 A | 03/09/97 |
| | | JP 10509309 T | 14/09/98 |
| | | NO 971945 A | 27/06/97 |
| | | NZ 296012 A | 28/05/99 |
| | | PL 319788 A | 01/09/97 |
| | | US 5618988 A | 08/04/97 |
| WO 9930701 A1 | 24/06/99 | AU 1897299 A | 05/07/99 |
| | | EP 1049460 A | 08/11/00 |
| | | NO 20003042 A | 14/06/00 |
| | | SE 511237 C | 30/08/99 |
| | | SE 9704693 A | 17/06/99 |
| WO 9837874 A1 | 03/09/98 | AU 719090 B | 04/05/00 |
| | | AU 2796797 A | 19/11/97 |
| | | AU 6295198 A | 18/09/98 |
| | | CN 1248912 T | 29/03/00 |
| | | EP 0898823 A | 03/03/99 |
| | | EP 0981338 A | 01/03/00 |
| | | NO 994109 A | 27/10/99 |
| | | PL 335370 A | 25/04/00 |
| | | SE 9700708 A | 28/08/98 |

## DNA construct and its use.

The present invention relates to a new DNA construct for transformation into oilseed plants. The DNA construct comprises nucleotide sequences encoding peptides with enzyme activities necessary for the high-level production and esterification of keto group-containing xanthophylls in oilseed plants.

### Background of the invention

Carotenoids are produced *de novo* by plants, fungi, algae and some bacteria. A number of biosynthetic steps are needed for the biological production of the carotenoids. There are two chemically different groups of carotenoids, namely carotenes containing only carbon and hydrogen molecules and xanthophylls containing oxygen in the molecule in addition to carbon and hydrogen.

The xanthophylls, and particularly astaxanthin (3,3'-dihydroxy-β-β-carotene-4,4'-dione), are often colored pigments and are used as such or as anti-oxidants.

Carotenes are biological precursors for the production of the oxygen-containing xanthophylls. There are two types of enzymes responsible for the introduction of hydroxy groups and keto groups into the carotenes, namely hydroxylases and ketolases, respectively.

The keto group-containing xanthophyll astaxanthin, which has keto and hydroxy groups, is biosynthetically produced from beta-carotene.

Large-scale production of xanthophylles from natural sources is at present performed by AstaCarotene AB, Gustavsberg, Sweden, by cultivation of the alga *Haematococcus pluvialis* for the production of astaxanthin in esterified form.

It would be desirable to be able to produce keto group-containing xanthophylls particularly astaxanthin, in oilseed plants. Oilseed plants have naturally β-carotene hydroxylases but lack β-carotene C-4-oxygenase enzymes or ketolases.

### Description of the invention

The present invention provides DNA constructs enabling and promoting production of keto group containing xanthophylls, especially astaxanthin, in oilseed plants, such as rape, sunflower, soybean and mustard. The DNA construct is transformed into the oilseed plant cell for expression of a protein or fused protein which has an enzyme activity enabling keto group insertion into a carotene or hydroxy carotene for the biosynthetic production of a keto group containing xanthophyll, such as cantaxanthin (β,β-carotene-4,4'-dione) and/or astaxanthin. Use is thus made of the biosynthetic pathway of the oilseed plant to

produce carotenoids. The naturally occurring synthesis of carotenoids involves a number of enzymes, namely 1-D-deoxyxylulose 5-phosphate synthase, isopentenyl pyrophosphate:dimethylallyl pyrophosphate isomerase, geranylgeranyl pyrophosphate synthase, phytoene synthase, phytoene desaturase, zeta-carotene desaturase, lycopene beta-cyclase, β-carotene hydroxylase, and β-carotene C-4-oxygenase. Genes coding for peptides having these enzymatic activities may be inserted into the DNA construct of the invention, one or several per construct, to promote high-level production in the transgenic oilseed plant. In case only one enzyme coding gene is inserted per plant, two or more plants may be sexually interbred to produce plants containing all the desired enzyme activities.

Thus, the present invention is directed to a DNA construct comprising in the 5′ to 3′ direction of transcription operably linked a promoter region directing transcription to the seed of an oilseed plant, a nucleotide sequence coding for at least one peptide with enzyme activity necessary for keto group containing xanthophyll production and esterification in an oilseed plant and a transcriptional termination region.

In a preferred embodiment of the invention the DNA construct additionally comprises between the promoter region and the nucleotide sequence coding for at least one peptide with enzyme activity a nucleotide sequence coding for a transit peptide directing the translated fusion polypeptide to the chloroplast of the oilseed plant.

The DNA construct is preferably such that the promoter is a napin promoter, the peptide with enzyme activity necessary for keto group containing xanthophyll production is selected from the group consisting of peptides with 1-D-deoxyxylulose 5-phosphate synthase, isopentenyl pyrophosphate:dimethylallyl pyrophosphate isomerase, geranylgeranyl pyrophosphate synthase, phytoene synthase, phytoene desaturase, zeta-carotene desaturase, lycopene beta-cyclase, β-carotene hydroxylase, and β-carotene C-4-oxygenase activity. To promote esterification of astaxanthin a nucleotide sequence coding for a peptide with acyl transferase activity may be included in the group.

In a preferred embodiment of the DNA construct according to the invention the nucleotide sequence coding for a peptide with enzyme activity is a nucleotide sequence coding for a N-terminally truncated β-carotene C-4-oxygenase gene from the alga *Haematococcus pluvialis*.

An example of the DNA construct of the invention is presented in the sequence listing as SEQ ID NO:1 and in Fig.1.

The present invention is also directed to a transgenic oilseed plant cell comprising the DNA construct of the invention, and preferably the oilseed plant is selected from the group consisting of rape, sunflower, soybean and mustard.

The invention is additionally directed to transgenic oilseed plant-produced xanthophyll, e.g. canthaxanthin and astaxanthin.

A preferred aspect of the invention is directed to transgenic oilseed plant-produced astaxanthin esters.

The present invention will now be illustrated with reference to the DNA construct disclosed in the sequence listing and in Fig.1, and the following description of embodiments. However, the invention is not limited to these exemplifications.

**Short description of the drawings**

Fig.1 illustrates the nucleotide sequence of the DNA construct comprising the napin promoter, the chloroplast localization signal, the N-terminally truncated β-carotene C-4-oxygenase gene and the termination sequence, and the deduced amino acid sequences of the transit peptide and the β-carotene C-4-oxygenase.

**Description of embodiments**

The invention is illustrated by production of astaxanthin in the seed of oilseed rape. The astaxanthin produced in the seed of the transgenic plant is extracted as part of the extracted oil. By use of conventionally used protocols for Agrobacterium tumefaciens mediated transformation such as described by (Hoekema et al.1983, An et al. 1986, Fry et al. 1987, DeBlock et al. 1988, Radke et al.1988, or Moloney et al. 1989) transgenic plants are produced having a chimeric DNA construct that is genetically inherited and is able to produce astaxanthin. The nucleotide sequence of the chimeric DNA construct consist of four parts of different genetic origin namely: (1) a promoter, (2) a localization signal, (3) a β-carotene C-4-oxygenase coding region and (4) a termination sequence.

The napin promoter directs transcription to the seed of oilseed rape (Stålberg et al 1996). This promoter was coupled to a localization signal similar but not identical to a transit peptide (TP) of Rbcs1a (Krebbers, 1988) that directs the translated product of a fused gene to the chloroplast. The promoter and the TP sequence were ligated to a part of the coding sequence of a ketolase gene BCK (Kajiwara et al. 1995). This enzyme oxygenates β-carotene to canthaxanthin, (Fraser et al. 1997). The chimeric DNA construct was then coupled to a suitable termination sequence, e.g. that of the Agrobacterium tumefaciens nopaline synthase gene (the nos 3´ end)(Bevan et al. 1983), as illustrated in Fig.1.

Cellular storage of Astaxantin

The storage of large amounts of free astaxanthin in plants will be difficult due to toxic effects of the molecule as it intercalates in the plant membranes. An effective esterification of astaxanthin to fatty acids enables storage of the esterified molecules in triacylglycerol containing oleosomes. Thus, an acyl transferase can be claimed to be of fundamental importance for the process, as is proteins that can mediate transport of different forms of astaxanthin from the chloroplast to the vesicles.

Sequences and oligonucleotides used in the construction of the DNA construct

*1. Napin promoter (GeneBank ACCESSION No. J02798)*

This promoter sequence, a 1145 base pair fragment including the 5' leader sequence has a unique HindIII site at the 5' end. The 3' end was synthesized with an additionally 6 nucleotide BamHI site.

*2. Transit peptide similar to RBCS1a (GeneBank ACCESSION No. X13611, X14565)*

The transit peptide (TP) was amplified by PCR from –28 to the end of the transit cleavage aa=54/55 site of the Rbcs1a gene. The 5' end was synthesized with a BamHI site and similarly the 3' sequence was synthesized with a XbaI site. The two following oligonucleotides were used for the PCR amplification.

BamHI

5' primer: TP1 5'AGAC **GGATCC** TCAGTCACACAAAGAGTA 3'

SacI XbaI

3' primer: TP2 5'GTTC **GAGCTC TCTAGA** CATGCAGTTAACGC 3'

*3. BCK (β-carotene C-4 oxygenase) (Genebank ACCESSION No. D45881)*

The BCK fragment was amplified by PCR including a 5' XbaI site and was ligated to the TP already described. The 5' primer (BCK1) used for PCR, is homologous to the BCK sequence from nucleotide 264 and the 3' oligonucleotide (Ax40) ends with a stop codon and was synthesized with a SacI restriction site for cloning. The synthesized fragment was fused to the TP as shown in Fig 1.

Oligonucleotides used for PCR:

XbaI

5' primer: BCK1 5'ACAG **TCTAGA** ATGCCATCCGAGTCGTCA 3'

SacI

3'primer: AX40 5'CACC**GAGCTC**CATGACACTCTTGTGCAGA 3'

**Description of SEQ ID NO:1 and SEQ ID NO:2**

The sequences shown i Fig.1 are the same as the two sequences which are shown in the sequence listing.

The SEQ ID NO:1 is a nucleotide sequence composed of the following features:

| | Nucleotide No. |
|---|---|
| Cloning site HindIII | 1-6 |
| Napin Promoter | 1-1145 |
| Cloning site BamHI | 1146-1151 |
| Transit peptide leader | 1152-1178 |
| Transit peptide coding | 1179-1347 |
| Cloning site XbaI | 1348-1353 |
| β-carotene C-4-oxygenase | 1354-2217 |
| β-carotene C-4-oxygense 3' untranslated | 2218-2266 |
| Cloning site SacI | 2267-2272 |
| Nopaline synthetase termination | 2273-2536 |
| Cloning site EcoRI | 2538-2543 |

The SEQ ID NO: 2 is a deduced amino acid sequence of the fusion protein of the transit peptide and the peptide with β-carotene C-4-oxygenase activity.

**References**

An G, Watson BD, Chiang CC (1986), Transformation of tobacco, tomato, potato and Arabidopsis-thaliana using a binary vector system. Plant Physiology 81 (1) 301-305.

Bevan M, BarnesWM and Chilton MD (1983). Structure and transcription of the nopaline synthase gene region of T-DNA. Nucleic Acids Res. 11 (2), 369-385 .

DeBlock M, DeBrouwer D, Tenning P (1989). Transformation of Brassica napus and Brassica oleracea using Agrobacterium tumefaciens and the expression of the BAR and NEO genes in transgenic plants Plant Physiology 91:2, 694-701.

Fraser PD, Miura Y, Misawa N, (1997). In vitro characterization of astaxanthin biosynthetic enzymes. J Biol Chem. Mar 7;272(10):6128-35.

Fry J, Barnason A, and Horsch RB, (1987). Transformation of Brassica napus with Agrobacteriium tumefaciens based vectors. Plant Cell Reports 6:321-325.

Hoekema A, Hirsch PR, Hooykas PJJ Schilperoort, (1983). A binary vector strategy based on separation of vir and T-region of the Agrobacterium tumefaciens Ti-plasmid. Nature vol 303, 179-180.

Josefsson LG, Lenman M, Ericson ML and Rask L, (1987). Structure of a gene encoding the 1.7 S storage protein, napin, from Brassica napus. J. Biol. Chem. 262 (25), 12196-12201.

Kajiwara S, KakizonoT, Saito T, Kondo K, OhtaniT, Nishio N, Nagai S and Misawa N. (1995). Isolation and functional identification of a novel cDNA for astaxanthin biosynthesis from Haematococcus pluvialis, and astaxanthin synthesis in Escherichia coli Plant Mol. Biol. 29 (2), 343-352.

Krebbers E, Seurinck J, Herdies L, Cashmore AR and Timko MP, (1988). Four genes in two diverged subfamilies encode the rubulose-1, 5-bisphosphate carboxylase small subunit polypeptides of Arabidopsis thaliana Plant Mol. Biol. 11, 745-759.

Moloney M, Walker JM and Sharma KK, (1989). High efficiency transformation of Brassica napus using Agrobacterium vectors. Plant Cell Reports 8:238-242.

Radke SE, Andrews BM, Moloney MM, Crouch ML, Kridl JC, Knauf VC (1988), Transformation of Brassica napus using Agrobacterium tumefaciens – Developmentally regulated Expression of a reintroduced napin gene. TAG, 75: (5) 685-694 .

Pua E-C, Mehra-Palta A, Nagy F and Chua N-H, (1987). Transgenic plants of Brassica napus. Biotechnology vol 5, 815-817.

Stålberg K, Ellerström M, Ezcurra I, Ablov S, Rask L (1996). Disruption of an overlapping E-box/ABRE motif abolished high transcription of the napA storage-protein promoter in transgenic Brassica napus seeds. Planta 199(4):515-9.

## Claims

1. A DNA construct comprising in the 5′ to 3′ direction of transcription operably linked a promoter region directing transcription to the seed of an oilseed plant, a nucleotide sequence coding for at least one peptide with enzyme activity necessary for keto group containing xanthophyll production and esterification in an oilseed plant and a transcriptional termination region.

2. The DNA construct according to claim 1, which between the promoter region and the nucleotide sequence coding for at least one peptide with enzyme activity additionally comprises a nucleotide sequence coding for a transit peptide directing the translated fusion polypeptide to the chloroplast of the oilseed plant.

3. The DNA construct according to claim 1 or 2, wherein the promoter is a napin promoter, the peptide with enzyme activity necessary for keto group containing xanthophyll production and esterification is selected from the group consisting of peptides with, 1-D-deoxyxylulose 5-phosphate synthase, isopentenyl pyrophosphate:dimethylallyl pyrophosphate isomerase, geranylgeranyl pyrophosphate synthase, phytoene synthase, phytoene desaturase, zeta-carotene desaturase, lycopene beta-cyclase, β-carotene hydroxylase, β-carotene C-4-oxygenase, and acyl transferase activity.

4. The DNA construct according to any one of claims 1 - 3, wherein the nucleotide sequence coding for a peptide with enzyme activity is a nucleotide sequence coding for a N-terminally truncated β-carotene C-4-oxygenase gene from the alga *Haematococcus pluvialis*.

5. The DNA construct according to claim 4, wherein the nucleotide sequence is SEQ ID NO:1.

6. Transgenic oilseed plant cell comprising the DNA construct of any one of claims 1-5 .

7. Transgenic oilseed plant cell according to claim 6, wherein the oilseed plant is selected from the group consisting of rape, sunflower, soybean and mustard.

8. Transgenic oilseed plant-produced xanthophyll.

9. Transgenic oilseed plant-produced xanthophyll according to claim 8, wherein the xanthophyll is canthaxanthin

10. Transgenic oilseed plant-produced xanthophyll according to claim 8, wherein the xanthophyll is astaxanthin.

11. Transgenic oilseed plant-produced xanthophyll according to claim 8, wherein the xanthophyll is astaxanthin esters.

<210> 2
<211> 346
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: deduced fusion protein of transit peptide + peptide with beta-carotene C-4 oxygenase activity

<400> 2

Met Ala Ser Ser Met Leu Ser Ser Ala Thr Met Val Ala Ser Pro Ala
1 5 10 15

Gln Ala Thr Met Val Ala Pro Phe Asn Gly Leu Lys Ser Ser Ala Ala
20 25 30

Phe Pro Ala Thr Arg Lys Ala Asn Asn Asp Ile Thr Ser Ile Thr Ser
35 40 45

Asn Gly Gly Arg Val Asn Cys Met Ser Arg Met Pro Ser Glu Ser Ser
50 55 60

Asp Ala Ala Arg Pro Ala Leu Lys His Ala Tyr Lys Pro Pro Ala Ser
65 70 75 80

Asp Ala Lys Gly Ile Thr Met Ala Leu Thr Ile Ile Gly Thr Trp Thr
85 90 95

Ala Val Phe Leu His Ala Ile Phe Gln Ile Arg Leu Pro Thr Ser Met
100 105 110

Asp Gln Leu His Trp Leu Pro Val Ser Glu Ala Thr Ala Gln Leu Leu
115 120 125

Gly Gly Ser Ser Ser Leu Leu His Ile Ala Ala Val Phe Ile Val Leu
130 135 140

Glu Phe Leu Tyr Thr Gly Leu Phe Ile Thr Thr His Asp Ala Met His
145 150 155 160

Gly Thr Ile Ala Leu Arg His Arg Gln Leu Asn Asp Leu Leu Gly Asn
165 170 175

Ile Cys Ile Ser Leu Tyr Ala Trp Phe Asp Tyr Ser Met Leu His Arg
180 185 190

Lys His Trp Glu His His Asn His Thr Gly Glu Val Gly Lys Asp Pro
195 200 205

Asp Phe His Lys Gly Asn Pro Gly Leu Val Pro Trp Phe Ala Ser Phe
210 215 220

Met Ser Ser Tyr Met Ser Leu Trp Gln Phe Ala Arg Leu Ala Trp Trp
225 230 235 240

Ala Val Val Met Gln Met Leu Gly Ala Pro Met Ala Asn Leu Leu Val
245 250 255

```
Phe Met Ala Ala Ala Pro Ile Leu Ser Ala Phe Arg Leu Phe Tyr Phe
            260                 265                 270

Gly Thr Tyr Leu Pro His Lys Pro Glu Pro Gly Pro Ala Ala Gly Ser
        275                 280                 285

Gln Val Met Ala Trp Phe Arg Ala Lys Thr Ser Glu Ala Ser Asp Val
    290                 295                 300

Met Ser Phe Leu Thr Cys Tyr His Phe Asp Leu His Trp Glu His His
305                 310                 315                 320

Arg Trp Pro Phe Ala Pro Trp Trp Gln Leu Pro His Cys Arg Arg Leu
                325                 330                 335

Ser Gly Arg Gly Leu Val Pro Ala Leu Ala
            340                 345
```

SEQUENCE LISTING

<110> AstaCarotene AB

<120> DNA construct and its use

<130> 29295-AstaCarotene

<140>
<141>

<160> 2

<170> PatentIn Ver. 2.1

<210> 1
<211> 2543
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: napin promoter
+ choroplast localization signal + beta-carotene C-4 oxygenase
coding sequence + termination sequence

<220>
<221> promoter
<222> (1)..(1145)

<220>
<221> transit_peptide
<222> (1179)..(1347)

<220>
<221> CDS
<222> (1179)..(2217)

<220>
<221> terminator
<222> (2273)..(2536)

<400> 1
aagctttctt catcggtgat tgattccttt aaagacttat gtttcttatc ttgcttctga 60

ggcaagtatt cagttaccag ttaccactta tattctggac tttctgactg catcctcatt 120

tttccaacat tttaaatttc actattggct gaatgcttct tctttgagga agaaacaatt 180

cagatggcag aaatgtatca accaatgcat atatacaaat gtacctcttg ttctcaaaac 240

atctatcgga tggttccatt tgctttgtca tccaattagt gactacttta tattattcac 300

tcctctttat tactattttc atgcgaggtt gccatgtaca ttatatttgt aaggattgac 360

gctattgagc gtttttcttc aattttcttt attttagaca tgggtatgaa atgtgtgtta 420

gagttgggtt gaatgagata tacgttcaag tgaagtggca taccgttctc gagtaaggat 480

gacctaccca ttcttgagac aaatgttaca ttttagtatc agagtaaaat gtgtacctat 540

```
aactcaaatt cgattgacat gtatccattc aacataaaat taaaccagcc tgcacctgca 600

tccacatttc aagtattttc aaaccgttcg gctcctatcc accgggtgta acaagacgga 660

ttccgaattt ggaagatttt gactcaaatt cccaatttat attgaccgtg actaaatcaa 720

ctttaacttc tataattctg attaagctcc caatttatat tcccaacggc actacctcca 780

aaatttatag actctcatcc ccttttaaac caacttagta aacgtttttt tttttaattt 840

tatgaagtta agtttttacc ttgtttttaa aaagaatcgt tcataagatg ccatgccaga 900

acattagcta cacgttacac atagcatgca gccgcggaga attgtttttc ttcgccactt 960

gtcactccct tcaaacacct aagagcttct ctctcacagc acacacatac aatcacatgc 1020

gtgcatgcat tattacacgt gatcgccatg caaatctcct ttatagccta taaattaact 1080

catccgcttc actctttact caaaccaaaa ctcatcaata caaacaagat taaaaacata 1140

cacgaggatc ctcagtcaca caaagagtaa agaagaaca atg gct tcc tct atg    1194
                                        Met Ala Ser Ser Met
                                         1               5

ctc tct tcc gct act atg gtt gcc tct ccg gct cag gcc act atg gtc   1242
Leu Ser Ser Ala Thr Met Val Ala Ser Pro Ala Gln Ala Thr Met Val
                10                  15                  20

gct cct ttc aac gga ctt aag tcc tcc gct gcc ttc cca gcc acc cgc   1290
Ala Pro Phe Asn Gly Leu Lys Ser Ser Ala Ala Phe Pro Ala Thr Arg
            25                  30                  35

aag gct aac aac gac att act tcc atc aca agc aac ggc gga cgc gtt   1338
Lys Ala Asn Asn Asp Ile Thr Ser Ile Thr Ser Asn Gly Gly Arg Val
        40                  45                  50

aac tgc atg tct aga atg cca tcc gag tcg tca gac gca gct cgt cct   1386
Asn Cys Met Ser Arg Met Pro Ser Glu Ser Ser Asp Ala Ala Arg Pro
    55                  60                  65

gcg cta aag cac gcc tac aaa cct cca gca tct gac gcc aag ggc atc   1434
Ala Leu Lys His Ala Tyr Lys Pro Pro Ala Ser Asp Ala Lys Gly Ile
70                  75                  80                  85

acg atg gcg ctg acc atc att ggc acc tgg acc gca gtg ttt tta cac   1482
Thr Met Ala Leu Thr Ile Ile Gly Thr Trp Thr Ala Val Phe Leu His
                90                  95                  100

gca ata ttt caa atc agg cta ccg aca tcc atg gac cag ctt cac tgg   1530
Ala Ile Phe Gln Ile Arg Leu Pro Thr Ser Met Asp Gln Leu His Trp
            105                 110                 115

ttg cct gtg tcc gaa gcc aca gcc cag ctt ttg ggc gga agc agc agc   1578
Leu Pro Val Ser Glu Ala Thr Ala Gln Leu Leu Gly Gly Ser Ser Ser
        120                 125                 130

cta ctg cac atc gct gca gtc ttc att gta ctt gag ttc ctg tac act   1626
Leu Leu His Ile Ala Ala Val Phe Ile Val Leu Glu Phe Leu Tyr Thr
    135                 140                 145
```

```
ggt cta ttc atc acc aca cat gac gca atg cat ggc acc ata gct ttg   1674
Gly Leu Phe Ile Thr Thr His Asp Ala Met His Gly Thr Ile Ala Leu
150                 155                 160                 165

agg cac agg cag ctc aat gat ctc ctt ggc aac atc tgc ata tca ctg   1722
Arg His Arg Gln Leu Asn Asp Leu Leu Gly Asn Ile Cys Ile Ser Leu
                170                 175                 180

tac gcc tgg ttt gac tac agc atg ctg cat cgc aag cac tgg gag cac   1770
Tyr Ala Trp Phe Asp Tyr Ser Met Leu His Arg Lys His Trp Glu His
            185                 190                 195

cac aac cat act ggc gaa gtg ggg aaa gac cct gac ttc cac aag gga   1818
His Asn His Thr Gly Glu Val Gly Lys Asp Pro Asp Phe His Lys Gly
        200                 205                 210

aat ccc ggc ctt gtc ccc tgg ttc gcc agc ttc atg tcc agc tac atg   1866
Asn Pro Gly Leu Val Pro Trp Phe Ala Ser Phe Met Ser Ser Tyr Met
    215                 220                 225

tcc ctg tgg cag ttt gcc cgg ctg gca tgg tgg gca gtg gtg atg caa   1914
Ser Leu Trp Gln Phe Ala Arg Leu Ala Trp Trp Ala Val Val Met Gln
230                 235                 240                 245

atg ctg ggg gcg ccc atg gca aat ctc cta gtc ttc atg gct gca gcc   1962
Met Leu Gly Ala Pro Met Ala Asn Leu Leu Val Phe Met Ala Ala Ala
                250                 255                 260

cca atc ttg tca gca ttc cgc ctc ttc tac ttc ggc act tac ctg cca   2010
Pro Ile Leu Ser Ala Phe Arg Leu Phe Tyr Phe Gly Thr Tyr Leu Pro
            265                 270                 275

cac aag cct gag cca ggc cct gca gca ggc tct cag gtg atg gcc tgg   2058
His Lys Pro Glu Pro Gly Pro Ala Ala Gly Ser Gln Val Met Ala Trp
        280                 285                 290

ttc agg gcc aag aca agt gag gca tct gat gtg atg agt ttc ctg aca   2106
Phe Arg Ala Lys Thr Ser Glu Ala Ser Asp Val Met Ser Phe Leu Thr
    295                 300                 305

tgc tac cac ttt gac ctg cac tgg gag cac cac aga tgg ccc ttt gcc   2154
Cys Tyr His Phe Asp Leu His Trp Glu His His Arg Trp Pro Phe Ala
310                 315                 320                 325

ccc tgg tgg cag ctg ccc cac tgc cgc cgc ctg tcc ggg cgt ggc ctg   2202
Pro Trp Trp Gln Leu Pro His Cys Arg Arg Leu Ser Gly Arg Gly Leu
                330                 335                 340

gtg cct gcc ttg gca tgacctggtc cctccgctgg tgacccagcg tctgcacaag   2257
Val Pro Ala Leu Ala
            345

agtgtcatgg agctcgaatt tccccgatcg ttcaaacatt tggcaataaa gtttcttaag 2317

attgaatcct gttgccggtc ttgcgatgat tatcatataa tttctgttga attacgttaa 2377

gcatgtaata attaacatgt aatgcatgac gttatttatg agatgggttt ttatgattag 2437

agtcccgcaa ttatacattt aatacgcgat agaaaacaaa atatagcgcg caaactagga 2497

taaattatcg cgcgcggtgt catctatgtt actagatcgg gaattc                 2543
```

Napin promoter

AAGCTTTCTTCATCGGTGATTGATTCCTTTAAAGACTTATGTTTCTTATCTTGCTTCTGA

GGCAAGTATTCAGTTACCAGTTACCACTTATATTCTGGACTTTCTGACTGCATCCTCATT

TTTCCAACATTTTAAATTTCACTATTGGCTGAATGCTTCTTCTTTGAGGAAGAAACAATT

CAGATGGCAGAAATGTATCAACCAATGCATATATACAAATGTACCTCTTGTTCTCAAAAC

ATCTATCGGATGGTTCCATTTGCTTTGTCATCCAATTAGTGACTACTTTATATTATTCAC

TCCTCTTTATTACTATTTTCATGCGAGGTTGCCATGTACATTATATTTGTAAGGATTGAC

GCTATTGAGCGTTTTTCTTCAATTTTCTTTATTTTAGACATGGGTATGAAATGTGTGTTA

GAGTTGGGTTGAATGAGATATACGTTCAAGTGAAGTGGCATACCGTTCTCGAGTAAGGAT

GACCTACCCATTCTTGAGACAAATGTTACATTTTAGTATCAGAGTAAAATGTGTACCTAT

AACTCAAATTCGATTGACATGTATCCATTCAACATAAAATTAAACCAGCCTGCACCTGCA

TCCACATTTCAAGTATTTTCAAACCGTTCGGCTCCTATCCACCGGGTGTAACAAGACGGA

TTCCGAATTTGGAAGATTTTGACTCAAATTCCCAATTTATATTGACCGTGACTAAATCAA

CTTTAACTTCTATAATTCTGATTAAGCTCCCAATTTATATTCCCAACGGCACTACCTCCA

AAATTTATAGACTCTCATCCCCTTTTAAACCAACTTAGTAAACGTTTTTTTTTTTAATTT

TATGAAGTTAAGTTTTTACCTTGTTTTTAAAAAGAATCGTTCATAAGATGCCATGCCAGA

ACATTAGCTACACGTTACACATAGCATGCAGCCGCGGAGAATTGTTTTTCTTCGCCACTT

GTCACTCCCTTCAAACACCTAAGAGCTTCTCTCTCACAGCACACACATACAATCACATGC

GTGCATGCATTATTACACGTGATCGCCATGCAAATCTCCTTTATAGCCTATAAATTAACT

CATCCGCTTCACTCTTTACTCAAACCAAAACTCATCAATACAAACAAGATTAAAAACATA

End -28 untranslated leader TP start

CACGAGGATCCTCAGTCACACAAAGAGTAAAGAAGAACAATGGCTTCCTCTATGCTCTCT

M A S S M L S

TCCGCTACTATGGTTGCCTCTCCGGCTCAGGCCACTATGGTCGCTCCTTTCAACGGACTT

S A T M V A S P A Q A T M V A P F N G L

AAGTCCTCCGCTGCCTTCCCAGCCACCCGCAAGGCTAACAACGACATTACTTCCATCACA

K S S A A F P A T R K A N N D I T S I T

FIG.1

TP End C-4-Oxygenase

```
AGCAACGGCGGACGCGTTAACTGCATGTCTAGAATGCCATCCGAGTCGTCAGACGCAGCT
 S  N  G  G  R  V  N  C  M  S  R  M  P  S  E  S  S  D  A  A

CGTCCTGCGCTAAAGCACGCCTACAAACCTCCAGCATCTGACGCCAAGGGCATCACGATG
 R  P  A  L  K  H  A  Y  K  P  P  A  S  D  A  K  G  I  T  M

GCGCTGACCATCATTGGCACCTGGACCGCAGTGTTTTTACACGCAATATTTCAAATCAGG
 A  L  T  I  I  G  T  W  T  A  V  F  L  H  A  I  F  Q  I  R

CTACCGACATCCATGGACCAGCTTCACTGGTTGCCTGTGTCCGAAGCCACAGCCCAGCTT
 L  P  T  S  M  D  Q  L  H  W  L  P  V  S  E  A  T  A  Q  L

TTGGGCGGAAGCAGCAGCCTACTGCACATCGCTGCAGTCTTCATTGTACTTGAGTTCCTG
 L  G  G  S  S  S  L  L  H  I  A  A  V  F  I  V  L  E  F  L

TACACTGGTCTATTCATCACCACACATGACGCAATGCATGGCACCATAGCTTTGAGGCAC
 Y  T  G  L  F  I  T  T  H  D  A  M  H  G  T  I  A  L  R  H

AGGCAGCTCAATGATCTCCTTGGCAACATCTGCATATCACTGTACGCCTGGTTTGACTAC
 R  Q  L  N  D  L  L  G  N  I  C  I  S  L  Y  A  W  F  D  Y

AGCATGCTGCATCGCAAGCACTGGGAGCACCACAACCATACTGGCGAAGTGGGGAAAGAC
 S  M  L  H  R  K  H  W  E  H  H  N  H  T  G  E  V  G  K  D

CCTGACTTCCACAAGGGAAATCCCGGCCTTGTCCCCTGGTTCGCCAGCTTCATGTCCAGC
 P  D  F  H  K  G  N  P  G  L  V  P  W  F  A  S  F  M  S  S

TACATGTCCCTGTGGCAGTTTGCCCGGCTGGCATGGTGGGCAGTGGTGATGCAAATGCTG
 Y  M  S  L  W  Q  F  A  R  L  A  W  W  A  V  V  M  Q  M  L

GGGGCGCCCATGGCAAATCTCCTAGTCTTCATGGCTGCAGCCCCAATCTTGTCAGCATTC
 G  A  P  M  A  N  L  L  V  F  M  A  A  A  P  I  L  S  A  F

CGCCTCTTCTACTTCGGCACTTACCTGCCACACAAGCCTGAGCCAGGCCCTGCAGCAGGC
 R  L  F  Y  F  G  T  Y  L  P  H  K  P  E  P  G  P  A  A  G

TCTCAGGTGATGGCCTGGTTCAGGGCCAAGACAAGTGAGGCATCTGATGTGATGAGTTTC
 S  Q  V  M  A  W  F  R  A  K  T  S  E  A  S  D  V  M  S  F

CTGACATGCTACCACTTTGACCTGCACTGGGAGCACCACAGATGGCCCTTTGCCCCCTGG
 L  T  C  Y  H  F  D  L  H  W  E  H  H  R  W  P  F  A  P  W
```

C-4 oxygenase Stop

```
TGGCAGCTGCCCCACTGCCGCCGCCTGTCCGGGCGTGGCCTGGTGCCTGCCTTGGCATGA
 W  Q  L  P  H  C  R  R  L  S  G  R  G  L  V  P  A  L  A  *
```

FIG.1 (cont.)

C-4 oxygenase untranslated region Nos term

CCTGGTCCCTCCGCTGGTGACCCAGCGTCTGCACAAGAGTGTCATGGAGCTCGAATTTCC

CCGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTG

CGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAAT

GCATGACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAAT

ACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCAT

end

CTATGTTACTAGATCGGGAATTC

Fig.1 (cont.)

(12) INTERNATIONAL APPLI[redacted]ON PUBLISHED UNDER THE PATENT C[redacted]ERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 March 2001 (22.03.2001)

PCT

(10) International Publication Number
WO 01/20011 A1

(51) International Patent Classification⁷: C12N 15/82, 9/02, 9/10, A01H 5/00, C12P 23/00 // (C12N 9/02, C12R 1:89)

(21) International Application Number: PCT/SE00/01767

(22) International Filing Date:
13 September 2000 (13.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9903336-7 17 September 1999 (17.09.1999) SE

(71) Applicant *(for all designated States except US)*: ASTACAROTENE AB [SE/SE]; Idrottsvägen 4, S-134 40 Gustavsberg (SE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: HÖGLUND, Anna-Stina [SE/SE]; Svankärrsvägen 27, S-756 53 Uppsala (SE). STÅLBERG, Kjell [SE/SE]; Vänortsgatan 118, S-752 63 Uppsala (SE).

(74) Agents: NILSSON, Brita et al.; AB Stockholms Patentbyrå, Zacco & Bruhn, Box 23101, S-104 35 Stockholm (SE).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published:**
— *With international search report.*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

WO 01/20011 A1

(54) Title: DNA CONSTRUCT AND ITS USE

(57) Abstract: A DNA construct comprising in the 5' to 3' direction of transcription operably linked a promoter region directing transcription to the seed of an oilseed plant, a nucleotide sequence coding for at least one peptide with enzyme activity necessary for keto group containing xanthophyll production and esterification in an oilseed plant and a transcriptional termination region is disclosed. The DNA construct may additionally comprise a nucleotide sequence coding for a transit peptide directing the translated fusion polypeptide to the chloroplast of the oilseed plant. The peptide with enzyme activity is preferably a peptide with β-carotene C-4-oxygenase activity, e.g. from the alga <*aematococcus pluvialis*. Comprised by the invention are also a transgenic oilseed plant cell, e.g. of rape, sunflower, soybean or mustard origin, and a transgenic oilseed plant-produced xanthophyll, such as canthaxanthin or astaxanthin, and also astaxanthin esters.